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(71) Applicant *(for all designated States except US)*: CORNELL RESEARCH FOUNDATION, INC. [US/US]; Suite 105, 20 Thornwood Drive, Ithaca, NY 14550 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: SMITH, Kendall, A. [US/US]; Apartment 3A, 1075 Park Avenue, New York, NY 10128 (US).

(74) Agents: AMZEL, Viviana et al.; Pretty, Schroeder & Poplawski, Suite 2000, 444 South Flower Street, Los Angeles, CA 90071 (US).

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(57) Abstract

A method of diagnosing a condition or disease associated with MICROBIAL infections, congenital or acquired immunodeficiencies, inflammatory, auto-immune, allergic, or dermatologic diseases, sarcoidosis, immunoscenesence, sepsis, necrosis, malignancies, or vaccine administration, comprises obtaining a sample comprising T or B cells from a subject suspected of being afflicted with the condition selected from the group consisting of conditions associated with infections by congenital or acquired immunodeficiencies, inflammatory, auto-immune, allergic, or dermatologic diseases, sarcoidosis, immunoscenesence, sepsis, necrosis, malignancies, or vaccine administration.

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# IDENTIFICATION OF ABNORMALITIES IN THE EXPRESSION OF T AND B CELL ANTIGEN RECEPTORS AS INDICATORS OF DISEASE DIAGNOSIS, PROGNOSIS AND THERAPEUTIC PREDICTORS

## BACKGROUND OF THE INVENTION

The present invention was made at least partially with Government funds under Grant Nos. R01-AI32031-20 and M01-RR00047. The US Government may have rights in this patent.

### Field of the Invention

This invention relates to the field of immune response by a mammal to challenges such as infectious microorganisms, particularly those by bacteria, fungi, parasites, and viruses, e.g., HIV, and to other conditions, hypothesized to be caused by a congenital or acquired immunodeficiency, inflammatory diseases, auto-immune diseases, sepsis, tissue necrosis, malignancies, the administration of vaccines, and the like. More specifically, the invention relates to a method and kit for assessing the establishment of specific patterns in which an animal reacts to infection, by rearranging or modifying its genetic make-up, in response to a specific challenge, and their application to the diagnosis and prognosis of disease, and for following the progress of therapy.

### Description of the Background

The immune system comprises three major types of lymphocytes: B cells, T cells, and natural killer (NK) cells. B-cells are derived from bone marrow, and comprise about 10% of the lymphocytes found circulating in blood. When stimulated by a specific antigen, each B cell differentiates into a plasma cell that secretes antibodies of a single specificity. T cells mature in the thymus and, make up about 80% of circulating lymphocytes. Although not producing antibodies, T cells bear on their surfaces specific antigen receptors resembling antibody molecules. T cells react to antigen stimulation by secreting immunomediator molecules or cytokines (helper T cells), and toxic molecules (cytotoxic T cells). Cytotoxic T cells act directly on infected cells, and by secreting toxic molecules kill them and any foreign particles, such as microorganisms, they may contain. NK cells, make up about 10% of the lymphocyte population, and are not antigen specific, but recognize and kill cells infected with microbes. Monocytes and macrophages are large scavenger cells that ingest foreign particles and present antigens to the T cells, which trigger specific immune responses. When an antigen is introduced, it is initially ingested by macrophages and other antigen presenting cells. After digestion, short segments thereof are presented on their cell surfaces. Only a few of all circulating T cells have receptors that specifically bind to the antigen, and this binding stimulates the T cells to secrete cytokines.

The immunodeficiency that occurs, for example, as a consequence of infection by microorganisms, such as the human immunodeficiency virus (HIV), results in a marked susceptibility

to infection by other microorganisms that would otherwise not invade the human body and cause disease, i.e., opportunistic infections (OI). This occurs with other conditions, which lower the organism's immune defense mechanism in one way or another, such as cancer therapy, bone marrow transplantation, and immunosuppressive therapy. These individuals, e.g., HIV+ individuals, become susceptible to OIs, for example, because of a progressive decline in the number of circulating CD4+ T lymphocytes (T cells), and also, presumably, because of a loss of previously primed CD4+ T cells or memory cells, which recognize foreign antigens of, e.g., common environmental microbes. Recent data obtained by the inventor indicate that antigen-primed CD4+ T cells are superior to unprimed CD4+ T cells (termed antigenically naive), in that primed CD4+ T cells are not only hypersensitive to antigen re-stimulation, which had already been shown, but they are also hyperreactive to antigen re-stimulation. Thus, primed T cells react to antigen re-stimulation by expressing genes encoding cytokines about 10 times as rapidly compared with unprimed, naive cells, and by secreting much greater quantities of cytokines, up to 100-fold higher amounts compared with unprimed naive cells. Since cytokines such as the interleukins, interferons, chemokines, and factors like lymphotoxin and tumor necrosis factor (TNF), are responsible for recruiting and activating the various white blood cells (WBCs or leukocytes) that mediate the immune/inflammatory response, a loss of primed, memory T cell CD4+ will result in a decline in the capacity of the immune system to respond to invasion by foreign microorganisms.

T cells recognize the presence of foreign antigens by means of cell surface structures which are termed T cell antigen receptors, or T cell receptor (TCR). The TCR is comprised of two membrane-spanning proteins of 4 types termed $\alpha$, $\beta$, $\gamma$, and $\delta$ chains. About 90% of T cells express the $\alpha$ and $\beta$ chains in heterodimeric pairs, with each T cell expressing a unique TCR comprised of distinct chains. At any given time, it is estimated that an individual expresses several million distinct TCRs. This marked diversity of the TCRs is encoded in the genome by several genomic gene segments known as the TCR V, D, J and C regions. Prior to expressing this genetic information, the TCR V, D, and J genomic segments rearrange and recombine, thereby creating the wide diversity of the TCR repertoire.

There are five major classes of antibodies or immunoglobulins in human blood plasma, IgG, IgA, IgM, IgD, and IgE, of which the IgG is far the most abundant. IgGs possess four polypeptide chains, two identical heavy (H) chains, of about 430 amino acid residues, and two identical light (L) chains, of about 214 residues. These chains are linked together by disulfide bonds into a Y-shape with flexible structure. Each chain has a region of constant amino acid sequence (C-region), and a region in which the sequence varies (V-region), which are bridged by a joining region (J-region). The antibody molecule has two binding sites for the antigen, with the VL and VH chains contributing to these binding sites. Each variable region comprises hypervariable regions, in which

there is an especially high frequency of amino acid replacement. These are the Complementarity Determining Regions (CDRs), which are in contact with their respective antigens. There are two types of L chains called kappa (κ) and lambda (λ), that have characteristic differences. Comparisons of the sequences of the L and H chains obtained from a number of myeloma patients have revealed characteristic similarities and variations in their antibodies. For instance, the carboxy-terminal portion of about 214 residues has essentially the same amino acid sequence in all patients, whereas the amino-terminal portion differs in sequence from one patient to another, although there are some homologies. The antibody chains appear to be coded for by duplication or repetition of primordial precursor genes, given the sequence homologies between the variable and constant halves of L chains and among the four segments of the H chains, each about 107 residues in length. There are, however, no sequence homologies between the VL chains and the VH chains. The clonal-selection hypothesis considers that all cells potentially capable of forming antibodies have the same genes, occurring in many different cell lines, called clones. Each of the clones makes only a single type of antibody with a characteristic amino acid sequence, with the genes for all other antibodies permanently repressed in this cell. In the absence of the antigen, each clone exists only in small numbers and makes only small amounts of its particular antibody, but it is committed to making that type of antibody even before it has seen that antigen. T cells cooperate in the formation of antibodies by interacting with a specific antigen. This interaction induces the T cells to release a factor that interacts with and activates those B cells that have also bound the antigen through specific cell-surface receptors. The activated B cells proliferate and some of their progeny become plasma cells which synthesize and secrete specific antibody. This occurs via a mechanism of rearrangement of the various regions, i.e., V, J, and C regions, into the final antibody molecule.

Individuals infected with infections, such as HIV, show an increased susceptibility to OIs, likely due to a loss of memory T cells reactive to common environmental microbes. In addition to the loss of CD4+ T cells, there may also be a qualitative change in the TCR repertoire within the time course of HIV infection of several years (average 8 years). Between the time of infection and the onset of the acquired immunodeficiency disease syndrome (AIDS), massive destruction of CD4+ T cells occurs, partially compensated for by the production of new T cells. On the order of 1-10 billion CD4+ T cells are destroyed and replaced daily. The destruction of the cells is likely due to effects of the virus itself, as well as to the immune response of the host to the viral antigens. The replacement of T cells is believed to result from either the maturation of new T cells from their precursors in the bone marrow and thymus, from the proliferative expansion of peripheral T cells, or both. However, given the dynamics of CD4+ T cell destruction and production that is constantly occurring in HIV+ individuals, it is often the case that abnormalities of the TCR repertoire occur, which ultimately result in a deficiency in the capacity to react to opportunistic organisms. Up

until the time of this invention, however, there existed no reliable methods of assessing, at the genetic level, either changes resulting from or associated with any of these diseases, the patient's prognosis, or the progress of therapy.

Thus, there is still a need for a method of assessing a pattern of genetic changes produced by a disease or condition associated with infection by a microorganism, auto-immune and other diseases, the prognosis for the patient's recovery or course of disease progression, and the progress of therapy by following changes in the patterns of T and B cell receptor changes (CR) or their chains, produced by the condition, or by a course of therapy. Such method permits an earlier detection of a condition or disease, and a more effective therapy course, as well as the alteration or combination of treatments to avoid, and/or ameliorate, symptoms, afflicting certain subjects with decreased immune response or an impaired immune system, in a safe manner, permitting them to resume an active life schedule.

## SUMMARY OF THE INVENTION

This invention relates to a method of diagnosing a condition or disease, which comprises

(a) obtaining a sample comprising T or B cells from a subject suspected of being afflicted with a condition selected from the group consisting of conditions associated with infections by congenital or acquired immunodeficiencies, inflammatory, auto-immune, allergic, or dermatologic diseases, sarcoidosis, immunoscenesence, sepsis, necrosis, malignancies, or vaccine administration;

(b) comprising reverse transcribing the sample's T or B cell receptor (TCR and BCR) mRNA fragments into cell receptor (CR) cDNA fragments prior to the determining step;

(c) determining the subject's most abundant length CR cDNA fragment, at least one of the CR cDNA fragments sequences, their length distribution or curve, and/or the area thereunder;

(d) comparing either the most abundant length CR cDNA fragment, at least one of the CR cDNA fragments sequences, their length distribution or curve, and/or area thereunder, to a library comprising either the most abundant length CR cDNAs, at least one of the CR cDNA fragment sequences, their length distributions or curves, areas thereunder, prognosis thereof, and/or diagnosis thereof, for all normal and diseased subject types of the same species, to determine any similarities; and

(e) diagnosing a disease or condition when either the patient's most abundant length CR cDNA fragment, at least one of the CR cDNA fragments sequences, their length distribution or curve, and/or area thereunder, is similar to either one of the most abundant length CR cDNA fragments, at least one of the CR cDNA fragments sequences, their length distribution or curve, and/or area thereunder, corresponding to a specific condition or disease comprised by the library.

This invention also relates to a method of assessing the prognosis of a subject afflicted

with a condition associated with infections, congenital or acquired immunodeficiencies, inflammatory, auto-immune, allergic, or dermatologic diseases, sarcoidosis, immunoscenesence, sepsis, necrosis, malignancies, and vaccine administration, comprising

(a) obtaining a sample comprising T or B cells from a subject afflicted with a condition selected from the group consisting of conditions associated with infections, congenital or acquired immunodeficiencies, inflammatory, auto-immune, allergic and dermatologic diseases, sepsis, tissue necrosis, sarcoidosis, immunoscenesence, malignancies, and vaccine administration;

(b) reverse transcribing the sample's T (TCR) or B (VCR) cell receptor (CR) mRNA into CR cDNA;

(c) determining the subject's most abundant length CR cDNA fragment, the CR cDNA fragments sequences, their fragments length distribution or curve, and/or area thereunder;

(d) comparing either the most abundant length CR cDNA fragment, at least one of the CR cDNA fragments sequences, their length distribution or curve, and/or area thereunder, to a library comprising either the most abundant length CR cDNA fragments, at least one of the CR cDNA fragments sequences, their length distributions or curves, area thereunder, diagnosis and/or prognosis thereof, obtained from all normal and diseased subject types of the same species, to determine any similarities;

(e) diagnosing a disease or condition when either the patient's most abundant length CR cDNA fragment, at least one of the CR cDNA fragments sequences, their length distribution or curve, and/or the area thereunder, is similar to either the most abundant length CR cDNA fragment, at least one of the CR cDNA fragments sequences, their length distribution or curve, and/or area thereunder, corresponding to a specific disease or condition, comprised by the library; and

(f) assessing a specific prognosis for the subject when, either the patient's most abundant length CR cDNA fragment, at least one of the CR cDNA fragments sequences, their distribution or curve, and/or the area thereunder, upon a comparison with the CR library, is found to be similar to either one of the most abundant length CR cDNA fragments, at least one of the CR cDNA fragments sequences, their length distribution or curve, and/or area thereunder, for a specific disease or condition CR cDNA subject type with a specific prognosis.

Still part of this invention is a method of assessing the progress of treatment administered to a subject afflicted with a condition associated with infections, congenital or acquired immunodeficiencies, inflammatory, auto-immune, allergic, or dermatologic diseases, sarcoidosis, immunoscenesence, sepsis, necrosis, malignancies, and vaccine administration, comprising

(a) obtaining samples comprising T or B cells from a subject afflicted with a condition selected from the group consisting of conditions associated with infection by a microorganism, congenital or acquired immunodeficiencies, inflammatory, auto-immune, allergic, or dermatologic

diseases, sepsis, necrosis, sarcoidosis, immunoscenesence, malignancies, and vaccine administration, prior to initiating treatment, and at specific time intervals thereafter;

(b) reverse transcribing the samples' T (TCR) or B (BCR) cell receptor (CR) mRNA into CR cDNA;

(c) determining the subject's most abundant length CR cDNA fragment, at least one of the CR cDNA fragments sequences, thier length distribution or curve, and/or area thereunder, prior to starting therapy and at the time intervals thereafter;

(d) comparing either the most abundant length CR cDNA fragment, at least one of the CR cDNA fragments sequences, their length distribution or curve, and/or area thereunder, obtained prior to initiation of the treatment with that (those) obtained at a later time interval (s), and to a CR cDNA library comprising either the most abundant length CR cDNA fragments, at least one of the CR cDNA fragments sequences, their length distributions or curves, and/or area thereunder, for all normal and diseased subject types from the same species, to determine any variations; and

(e) assessing an effective treatment when, as time progresses, either the patient's most abundant length CR cDNA fragment, at least one of the cDNA fragments sequences, their distribution or curve, and/or area thereunder, becomes more similar to either the most abundant length CR cDNA, at least one of the cDNA fragments sequences, their length distribution or curve, and/or area thereunder, for the corresponding normal CR cDNA subject type or less similar to that obtained prior to initiation of treatment or to the CR cDNA for the specific condition or disease.

This invention also relates to diagnostic kits for assessing a subject's condition or disease, and determining prognosis and therapy, which comprise a library of all normal and diseased subject types of either the most abundant length CR cDNA fragment, at least one of the CR cDNA fragments sequences, their length distribution or curve, area thereunder, diagnosis thereof, and/or prognosis thereof, and instructions for its use. The diagnostic kit may also contain the necessary reactants for determining cDNA sequences from a sample and comparing them to the library of standards.

## BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the 24 TCR B CDR3 size (cDNA length) patterns, BV1-BV24, utilizing T cells from a normal healthy adult. There is a normal Gaussian distribution of the CDR3 fragments lengths, ranging from 8 to 13 amino acid residues, with an average of 10.

Figure 2 shows the 24 TCR B CDR3 size patterns from Patient No. 3. There are abnormal oligoclonal patterns for BV4, BV6, BV7, and BV10, and monoclonal patterns for BV11 and BV24.

Figure 3 shows the 24 TCR B CDR3 size patterns from Patient No. 8. There are abnormal, monoclonal patterns for BV10, BV22, and BV24.

Figure 4 shows the 24 TCR B CDR3 size patterns from Patient No. 9. There are abnormal, oligoclonal patterns for BV5, BV9, BV13 and BV22, and monoclonal patterns in BV24.

Figure 5 shows the 24 TCR B CDR3 size pattern from Patient No. 15. There are monoclonal patterns for BV14, BV22, and BV24, and oligoclonal patterns for BV6, BV10, BCV14, BV15, BV16, and BV21.

## DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention arose from a desire by the inventor to improve on prior art technology. In the past, diagnosis, prognosis, and therapy had been attempted on patients afflicted with diseases or conditions associated with microbial infections, congenital or acquired immunodeficiencies, inflammatory, auto-immune, allergic, or dermatologic diseases, sarcoidosis, immunoscenesence, sepsis, necrosis, malignancies, and vaccine administration, among others, without the benefit of reliable indicators. Many of these diseases are believed to be caused either by direct infection, or indirectly as a result of a decrease in immune response, as, for example, in HIV+ patients, in patients suffering from some forms of cancer, and even in patients that have been subjected to bone marrow transplant (BMT), and other chronic inflammatory, skin, and metabolic diseases, sepsis, auto-immune diseases, and the like. In the past, the diagnosis, assessment of prognosis, and therapy were conducted solely by relying on the symptoms or relief thereof to adjust dosage and assess the effectiveness of a specific treatment, without the benefit of a marker for assessing the presence of the condition of disease, e.g., microbial infection, for assessing the prognosis and/or following a course of therapy.

This invention permits the correlation of changes in protein, RNA, and DNA patterns, with disease and toxic side effects, for example, in the case of high doses of cytokine therapy administered to HIV+ subjects which bring about extremely severe side effects, such as those classified by the World Health Organization (WHO) as toxicities of Grade 1 to Grade 4.

This invention, thus, permits the adjustment of a course of therapy or other treatments with, for example, anti-microbials, or other drugs, such as cytokines, administered to HIV+ subjects, while adjusting the dosages of the therapeutic agents to avoid producing toxic side effects detrimental to the well being and/or life style of the patient. This also permits the administration of therapy for prolonged periods of time without discontinuance, or masking any undesirable effects of the drug. The present method is applicable to the assessment of a condition or disease, to the prognosis of the course of a condition, and for following a course of therapy in symptomatic and asymptomatic individuals for prolonged periods of time, even several years, while avoiding toxic side effects.

The method and diagnostic kits of this invention are useful for the various applications described above, including the diagnosis of a condition or disease, the assessment of the prognosis for the condition or disease, and for following the progress of the disease when the subject is

administered a specific course of therapy. The conditions of diseases for which the present method and kit are well suited are those associated with infection by specific microorganisms, which directly or indirectly cause the disease or condition, congenital or acquired immunodeficiencies, chronic inflammatory diseases, auto-immune diseases, allergic diseases, chronic dermatologic diseases, sepsis, necrosis, all types of malignancies or cancers, sarcoidosis, immunoscenesence, and vaccine administration, among other groups. Some of the diseases for which the present technology is best suited are not well characterized, and at one time or another were believed to belong to the auto-immune, inflammatory, infectious disease category, were generally classified as not well characterized. Some of the diseases for which the present technology is best suited are chronic inflammatory diseases, e.g., inflammatory bowel disease, such as crohn's disease, and ulcerative colitis, pancreatitis, cholecystitis, immune-mediated glomerulonephritis, rheumatoid arthritis, systemic lupus erythematosus, scleroderma, myositis, allergies, such as allergic rhinitis, asthma, and anaphylaxis, polyarteritis, psoriasis, allergic dermatitis, and chronic urticaria, among others. This technology is also applicable to all types of cancers, such as, carcinomas, sarcomas, leukemias, lymphoma, myelomas, among others, to a variety of infectious diseases, such as bacterial infections, mycobacterial infections, spirochetal infections, fungi infections, rickettsial infections, mycoplasmal infections, chlamydia infections, viral infections, and parasitic infections, among others. The present technology is also applicable to other diseases, such as sarcoidosis, diabetes, Grave's disease-hyperthyroidism, multiple sclerosis, immunoscenesence, among many.

**Methods for Analyzing TCR and BCR Repertoire**

Since the discovery of the T cell receptor (TCR) and the B cell bound antibodies and their elucidation at the cellular, protein and genetic levels in the 1970s and 1980s, various attempts have been made to examine the diversity and complexity of all of the TCRs and BCRs expressed, or studying the so-called TCR and BCR repertoire. Although many attempts have been made, up to the present time there is no reliable and effective method which can dissect the overall structure of the TCR at the molecular level. At the cellular level, however, individual T cell and B cell clones expressing only a single TCR have been established. The frequency of specific antigen-reactive cells has been determined by limiting dilution analysis. A limited attempt to study the frequency of expression of the various types of V-regions has been made with the aid of monoclonal antibodies. V-region specific monoclonal antibodies, however, are only available for about half of the total number of the V-region gene products, and this precludes the analysis of all of the TCR repertoire.

The genes or mRNA transcripts encoding the TCR have been studied using molecular methods, such as by the RNAse protection assay or by the polymerase chain reaction (PCR), additionally with a DNA sequencer to analyze the TCR repertoire, and allows the characterization of a TCR transcript not only by its V-gene region, but also by its J-region along the length of its

Complimentarily Determining Region 3 (CDR3). The BCR has been studied structurally, particularly in abnormalities associated with various forms of cancers. A similar application is made herein to the BCR. The CDR3 is particularly important in both the TCR and BCR, because this region comprises the VDJ region re-arrangement, and encodes the portion of the TCR protein and BCR antibody that is especially involved in contacting the antigen binding the TCR or BCR.

The TCR and BCR repertoires, thus, may be analyzed at the mRNA level. For this, the mRNA is extracted from, e.g., isolated peripheral blood cells, such as T and B cells, reverse transcribed into cDNA, and then amplified using specific primers, e.g., a panel of V-region specific primers together with a C-region specific primer. By means of example, the following information is provided in reference to the B chain of the TCR, but similar application may be made to the A ($\alpha$), C ($\gamma$), and D ($\delta$) chains of the TCR, and the L and H chains of the BCR. Briefly, the amplifications may be carried out to saturation (40 cycles), and aliquots of the amplified material may be copied in run-off reactions, initiated by a third, nested, fluorescent dye-labeled BC, BJ, or clonotypic-specific primer. The labeled DNA strands may then be analyzed by an automated DNA sequencer, and the length of the fluorescent profiles analyzed by computer to determine the size (length), and/or area of each DNA peak. This analysis allows the detection of expressed V-regions as well as the absence of V-regions that are not expressed at all, suggestive of clonal deletion, and whether or not there is skewing of expression of one or several CDR, e.g., CDR3, lengths. The latter is indicative of the selection of several clones (oligoclonal) or a single clone (monoclonal), and generally reflects the selection of a TCR or BCR reactive to a particular antigen.

This invention, therefore, provides a method of diagnosing a condition or disease associated with microorganism infections, congenital or acquired immunodeficiencies, inflammatory diseases, auto-immune diseases, allergic diseases, dermatologic diseases, sarcoidosis, immunoscenesence, sepsis, necrosis, malignancies, and vaccine administration, among others, which generally comprises the following steps

(a) obtaining a sample comprising T or B cells from a subject suspected of being afflicted with a condition selected from the group consisting of conditions associated with microbial infections by congenital or acquired immunodeficiencies, inflammatory, auto-immune, allergic, or dermatologic diseases, sarcoidosis, immunoscenesence, tissue sepsis, necrosis, malignancies, or vaccine administration;

(b) reverse transcribing the sample's T or B cell receptor (TCR and BCR) mRNA fragments into cell receptor (CR) cDNA fragments prior to the determining step;

(c) determining the subject's most abundant length CR cDNA fragment, at least one of the CR cDNA fragments sequences, their length distribution or curve, and/or the area thereunder;

(d) comparing either the most abundant length CR cDNA fragment, at least one of the CR

cDNA fragments sequences, their length distribution or curve, and/or area thereunder, to a library comprising either the most abundant length CR cDNAs, at least one of the CR cDNA fragment sequences, their length distributions or curves, areas thereunder, prognosis thereof, and/or diagnosis thereof, for all normal and diseased subject types of the same species, to determine any similarities; and

(e) diagnosing a disease or condition when either the patient's most abundant length CR cDNA fragment, at least one of the CR cDNA fragments sequences, their length distribution or curve, and/or area thereunder, is similar to either one of the most abundant length CR cDNA fragments, at least one of the CR cDNA fragments sequences, their length distribution or curve, and/or area thereunder, corresponding to a specific condition or disease comprised by the library.

This method may be conducted by utilizing the original RNA transcript, rather than reverse transcribing it into cDNA. Because of the susceptibility of RNAs to degradation, however, it is generally more practical to utilize the reverse transcribed cDNA for laboratory manipulation. This is true even when complete automation is desired, including all steps other than a sample procurement from an individual to be screened, and loading into an analyzer. Thus, although this technology may be developed at the messenger RNA level, it will be described herein by reference to cDNA, and it is clearly applicable to either one.

The library may contain information on standards in various forms, including distribution or curves, areas, information on the most abundant length TCR or BCR cDNAs, and/or sequences thereof. This information may be provided for all normal subject types of one species, of several species, from normal and diseased individuals. Moreover, the libraries may contain patterns of TCR or BCR cDNAs from individuals afflicted with one or another condition or disease, or with multiple conditions of diseases. Clearly, when the screening of a population is desired, it may be desirable to utilize a more encompassing library in order to detect patterns representative of conditions or diseases, such as in the case of infections by microorganisms.

The samples may be liquid or solid, and must contain peripheral lymphocytes, such as blood, serum, or lymphatic fluid, among others. The present method is suitably utilized in animals, e.g., mammals, including domesticated and wild animals, such as humans, canines, equines, ovines, felines, pigs, sheep, simians, and the like. The method, however, may be applied to other animals, as well. Infections which affect animals are well known, including scrapies, mad cow disease, SIV, and the like, in addition to other infections and diseases and conditions which are also common to humans. Animals are also afflicted with a variety of cancers such as leukemia, inflammatory diseases, auto-immune diseases, and the like, and are vaccinated against many diseases, as well. The present technology is, thus, applicable to subjects that are afflicted with a disease or condition associated with bacterial, viral, fungal, mycoplasmic, and parasitic microorganisms,

congenital or acquired immunodeficiencies, auto-immune diseases, allergic diseases, inflammatory diseases, dermatologic diseases, immunoscenescence, sarcoidosis, sepsis, necrosis, or malignancies, or are administered a vaccine, among others. This invention is particularly suitable for the determination of infection by a virus, such as HIV, whether the subject is HIV seropositive or negative.

In one of the embodiments of the invention, the work of the cDNA materials is conducted as described by Pannetier et al., Immunology Today 4: 176-181 (1995). Briefly, the transcribed TCR or BCR cDNA may be separated amplified by the polymerase chain reaction (PCR) with unlabeled primers complementary to regions vicinal to the complementarity determining regions (CDR) of the T cell receptor (TCR) or BCR up- and down-stream thereto, under conditions which permit full saturation. Thus obtained unlabeled cDNA may then be amplified by PCR with one additional primer, which is labeled, under conditions effective to attain a run-off reaction elongating the amplified cDNA over the vicinal CDR region. Thereafter, the cDNA label with the first label, may be separated and analyzed by comparison to normal and diseased samples. The amplification step may be separately repeated with different primers and labels, and the differently labeled cDNAs may be separated, e.g., on a gel, such as by electrophoresis, for analysis. The primers utilized are generally V, and C or J, and in the case of the exemplary disclosure BV, BJ, and BC. However, others may also be utilized. Preferably, the CDR comprises a CDR3 region. Pairs of primers which may be utilized are from the V- and C-regions, the V- and J-regions, or both. In a very desirable embodiment, the analysis of the data by comparison to the standards in the library is computerized, and more preferably the entire procedure is automated, with the only manual manipulation being obtaining a sample from a subject.

In similar manner, this method of diagnosing a disease or condition may be conducted by comparing the cDNA sequences of the highest profile peak, or of several peaks obtained for all normal and diseased subject types. This may be automated and compared with the data in the cDNA sequence library manually or with the aid of a computer, and specially tailored software.

The latter method relies on specific sequences, which are characteristic of T cell or B cell receptors, cDNA sequences obtained from subjects which are normal and either free of, or afflicted by a specific condition or disease. Thus, whereas the method described above relies on cDNA length patterns, this method relies on the specific RNA or DNA sequences of either one or multiple peaks present in a subject's sample and compares it (them) to standards in a library. A preferred method relies on the sequence of the most abundant length TCR or BCR cDNA sequences and compares it (them) to the sequences present in the library. TCR and/or BCR sequences or length distribution patterns or areas under the curves delimiting the patterns, however, may also be utilized.

Both of the methods described above may also be utilized for assessing the prognosis

of a subject afflicted by one or more of the diseases or conditions described above by

(a) obtaining a sample comprising T or B cells from a subject afflicted with a condition selected from the group consisting of conditions associated with microbial infections, congenital or acquired immunodeficiencies, inflammatory, auto-immune, allergic, or dermatologic diseases, sepsis, tissue necrosis, malignancies, immunoscenesence, sarcoidosis, and vaccine administration;

(b) reverse transcribing the sample's T (TCR) or B (VCR) cell receptor (CR) mRNA into CR cDNA;

(c) determining the subject's most abundant length CR cDNA fragment, at least one of the CR cDNA fragments sequences, their fragment length distribution or curve, and/or area thereunder;

(d) comparing either the most abundant length CR cDNA fragment, at least one of the CR cDNA fragments sequences, their length distribution or curve, and/or area thereunder, to a library comprising either the most abundant length CR cDNA fragments, at least one of the CR cDNA fragments sequences, their length distributions or curves, area thereunder, diagnosis thereof, and/or prognosis thereof, obtained from all normal and diseased subject types from the same species, to determine any similarities;

(e) diagnosing a disease or condition when either the patient's most abundant length CR cDNA fragment, at least one of the CR cDNA fragments sequences, their length distribution or curve, and/or the area thereunder, is similar to either the most abundant length CR cDNA fragment, at least one of the the CR cDNA fragments sequences, their length distribution or curve, and/or area thereunder, corresponding to a specific disease or condition, comprised by the library; and

(f) assessing a specific prognosis for the subject when, either the patient's most abundant length CR cDNA fragment, at least one of the CR cDNA fragments sequences, their distribution or curve, and/or the area thereunder, upon a comparison with the CR library, is found to be similar to either one of the most abundant length CR cDNA fragments, at least one of the CR cDNA fragments sequences, their length distribution or curve, and/or area thereunder, for a specific disease or condition CR cDNA subject type with a specific prognosis.

This can be attained by either relying on the cDNA sequences of one or more of the TCR or BCR cDNA peaks, or the subject's most abundant length TCR or BCR cDNA fragment, the CR cDNA fragments' length distribution or curve, and/or the area thereunder, and comparing either one to the corresponding standards stored in a library, which additionally contains information on the diagnosis, and prognosis asociated with specific markers obtained from subjects which, for example, developed the full-blown disease in a short time or never at all, attained a repression or full recovery, and the like.

The present methods may also be applied to the assessment of progress of the

treatment of one of the conditions or diseases discussed, generally comprising

(a) obtaining samples comprising T or B cells from a subject afflicted with a condition selected from the group consisting of conditions associated with infection by a microorganism, congenital or acquired immunodeficiencies, inflammatory, auto-immune, allergic, or dermatologic diseases, sepsis, tissue necrosis, sarcoidosis, immunoscenesence, malignancies, and vaccine administration, prior to initiating treatment, and at specific time intervals thereafter;

(b) reverse transcribing the samples' T (TCR) or B (BCR) cell receptor (CR) mRNA into CR cDNA;

(c) determining the subject's most abundant length CR cDNA fragment, at least one of the CR cDNA fragments sequences, thier length distribution or curve, and/or area thereunder, prior to starting therapy and at the time intervals thereafter;

(d) comparing either the most abundant length CR cDNA fragment, at least one of the CR cDNA fragments sequences, their length distribution or curve, and/or area thereunder, obtained prior to initiation of the treatment with that (those) obtained at a later time interval (s), and to a CR cDNA library comprising either the most abundant length CR cDNA fragments, at least one of the CR cDNA fragments sequences, their length distributions or curves, and/or area thereunder, for all normal and diseased subject types from the same species, to determine any variations; and

(e) assessing an effective treatment when, as time progresses, either the patient's most abundant length CR cDNA fragment, at least one of the cDNA fragments sequences, their distribution or curve, and/or area thereunder, becomes more similar to either the most abundant length CR cDNA, at least one of the cDNA fragments sequences, their length distribution or curve, and/or area thereunder, for the corresponding normal CR cDNA subject type or less similar to that obtained prior to initiation of treatment or to the CR cDNA for the specific condition or disease.

The methods of the invention may be applied by using kits for either diagnostic purposes, for assessing the prognosis of a subject afflicted with the condition or disease, and/or for following the course of a specific therapy. The kit may contain a library comprising cDNA standards for all normal and diseased subject types, and instructions for its use. The kit may also contain reverse transcriptase, and PCR reagents and other necessary reactants, such as enzumes, labels, primers, and the like, suitable for RNA transcriptoin and/or DNA amplification and/or sequencing. The library may be in computerized form, and the standards may be either at least the most abundant length TCR and BCR cDNA fragments, or all of them, at least one of the CR cDNA fragments sequences, their length distributions or curves, or the areas thereunder, for all normal and diseased subject types. In different embodiments, the library may comprise T cell and B cell receptor cDNA sequences, preferably the most abundant length T cell or B cell receptor cDNAs, and still more preferably the T cell or B cell receptor V-region cDNAs, and still more preferably the TCR A, B,

C or DV-regions or the BCR VL or HL regions, or their CDR regions, e.g., the CDR3 regions. The library may contain standards for one or more species, and for one or a plurality of diseases, or standards from subjects afflicted with more than one disease. When the kit is intended for determining DNA sequences, it may further comprise PCR reagents, and/or one or more DNA labeling materials, such as phosphorescent, radioactive, and/or fluorescent labels. When the kit is intended for the determining of cDNA lengths, it may also include primers for regions vicinal to the desired V-region, e.g., the CDRs, and preferably CDR3s.

The present technology may be applied to humans as well as all types of animals, including equines, bovines, ovines, large and small animals in the care of a veterinarian, and animals found in the wild, among others. In fact, the present invention extends to the screening of populations, the testing of normal subjects, for example in cases of individual travel to areas of the country or the world affording the possibility of a contagious infection, and the like. The present invention is also applicable to the detection and follow-up of treatment of viral, bacterial, fungal and parasitic infections in general, congenital or acquired immunodeficiencies, inflammation, necrosis, sepsis, and cancers or other malignancies, such as carcinomas, melanomas, sarcomas, leukemias, lymphomas, and myelomas, as well as a follow-up to vaccine administration, among others, as discussed above.

In the case of viral infections, particularly in the presence of the human immunodeficiency virus, one of the events that prevents therapy with specific drugs is the observation of an increase in the circulating viral count of the individual. This, in turn, prompts either an adjustment of the dose of the drug being administered to the individual or a complete stoppage of the therapy. The present invention, thus, permits the assessment of the changes that may occur at the transcription and expression levels as a consequence of disease or therapy, or both. This invention permits the follow-up of therapies administered as the main therapy in the indicated types of infections, or after other interventions, such as surgery, chemotherapy or radiotherapy. In the case of HIV+ or AIDS patients, the present invention permits the assessment of progress with therapies that may encompass a main therapeutic agent, and combined therapies, such as with interleukins, nucleic acid analogues, other anti-viral drugs, and enzyme inhibitors, and some combined therapies which include, for example, one or more anti-viral agents of the nucleic acid analogue class and one or more protease inhibitors, and/or cytokines. More generally, the present invention is useful to follow the progress of individuals after the administration of vaccines, such as hepatitis B and C vaccines, and the like, with or without adjuvants, including cytokines administered in the same formulation with these agents or by a different route. This invention permits, as well, the assessment of alternating the administration of different drugs, alone or in combination with another therapy being administered over a prolonged period of time. Also useful is the application of the present

invention, when specific drugs for which there is no established dose in the art are combined, the effect of the combined therapy may be followed, by starting with a lower dose of each individual drug and then progressively increasing their doses one at a time while monitoring the effect produced on one of more of the parameters followed, as described above. An artisan with average skill in the art of medicine or veterinary science would know how to proceed to attain a desirable combination dose for a specific therapy.

The present invention is applicable whether the therapy is self-administered by the human subject or by a health professional, whether administered subcutaneously, transdermally, intrapulmonarily, transbuccally, or by implant. The present invention permits the balancing of favorable and unfavorable aspects of a specific therapy. For example, the administration of an agent such as cytokine, may produce an increase in one or more of a group of circulating immunity-building cells, such as lymphocytes, monocytes and polymorphonuclear lymphocytes, including T cells, particularly CD4+ T cells, B cells, natural killer (NK), eosinophils, monocytes, basophils, or antigen-presenting cells. The administration of this agent may also produce a detrimental genetic effect by altering the T cell or B cell receptor V-region profile or curve or the area under it, or the sequence of the main component (s) of the TCR or BCR receptor V-region cDNA. This invention may, thus, help in selecting one or another treatment, or in determining a dose which favorably impacts the treated individual while producing some of the functionally desirable effects, as well.

Examples of therapies that may be aided by the present invention are cytokines, which may be administered individually or jointly with other cytokines from each of the four listed groups, and/or other drugs. The therapy may encompass the administration of 2, 3 and more of the IL-2 to 15 interleukins, alone or in combination with other cytokines, antivirals, anti-inflammatories, etc., one or more of the interleukins with one or more tumor necrosis factors (TNFs), one or more members of the TNF family, such as a TNF-α, NGF, and one or more members of the interferon or interleukin families, a member of the chemokine family such as IL-8, MIP or Rantes, and one or more members of one of the other families, and the like, including all combinations of different cytokines, their fragments, analogues and derivatives, and mixtures thereof. The therapy assessed by the present method may also comprise one or more antiviral agents, such as any agents utilized in the treatment of viral infections. Examples are zidovudine (AZT), 2',3'-dideoxyinosine (ddI), 3'-azido-2', 3'-dideoxythymidine, acyclovir, 1, 3-dihydro- 2-propoxy-methyquanine (gancyclovir), ribavirin, dideoxycytidine (ddC), lamivudine (3TC), enzyme inhibitors, such as protease inhibitors, e. g., sequinovir, ritonavir, and indinovir, among others, and combinations thereof in twos, threes, and higher numbers, including combinations of one or more nucleic acid analogues and one or more protease inhibitors with one or more cytokines, one or more cytokine(s) and three antiviral compounds, such as 3TC, acyclovir, ritonavir, sequinovir, and indinovir, among others. The anti-

bacterial therapy may be with any agent known to be effective against the agent associated with the infection. Examples of anti-bacterial therapies are pentamidines, trimethoprim-sulfamethoxazole, sulfonamides, penicillins, cephalosporins, aminoglycosides, tetracyclines, chloramphenicols, and combinations thereof, as well as those anti-microbial drugs delivered by specifically targeted antibodies and their fragments which are attached to them at specific sites. Examples are radioisotope, enzyme, toxin, and other therapeutic agent-carrying monoclonal antibodies, and combinations thereof. Moreover, the present technology may be utilize to follow the course of infection with, and/ or therapy against, any fungi. Examples of anti-fungal therapies that may be aided by this invention are flucytosine, amphotericin B, fluconazole, griseofulvine, and combinations thereof. Similarly, this invention is applicable to therapies conducted with any anti-parasitic agent. Examples agents are pyrimethamine, quinacrine, thiabendazole, levamisol, and combinations thereof. Therapies with any anti-metabolic agent may be followed with the present technology, as well. Examples of therapies are those with are purine analogues, folic acid analogues, pyrimidine analogues, and combinations thereof, among others. This method permits the assessment of any anti-inflammatory therapy, alone or in combination with other drugs or treatments. Examples are steroidal anti-inflammatory agents, non-steroidal anti-inflammatory agents such as acetaminophen and aspirin, and combinations thereof, alone or with other drugs or treatments. This method may also be applied to additional drugs being administered, such as vasoactive agents such as epinephrine, norepinephrine, dopamine and combinations thereof, vaccines such as hepatitis B and C vaccines, bronchodilating agents such as $\beta_2$ receptor agonists, and combinations thereof, local anesthetic agents such as procaine, cocaine, and combinations thereof, growth promoting and regenerating agents such as epidermal growth factor, fibroblast growth factor, and combinations thereof, additional lymphokines or cytokines such as interleukins other than IL-2, interferons, and the like, hematopoietins, growth factors, hormones, chemokines, active analogues, fragments, fusion proteins and pharmaceutically-acceptable derivatives thereof, and combinations thereof, agents such as soluble CD4 and analogues thereof, anionic polysaccharides, and anti-neoplastic agents such as alkylating agents, anti-metabolites, hormones, vinca, alkaloids, anti-proliferative agents, and combinations thereof. Therapies with other bioactive agents of similar or different activities and applications are also encompassed herein.

Having now generally described the invention, the same will be better understood by reference to certain specific examples, which are included herein for purposes of illustration only, and are not intended to be limiting of the invention or any embodiment thereof, unless so specified.

## EXAMPLES

**Example 1: Preparation of Subject's Sample and cDNA**

To analyze the TCR or BCR repertoire of individuals infected with HIV, blood samples were collected from 16 subjects known to be HIV seropositive and who had CD4+ T cell counts in the range of 200-500 cells/$mm^3$ blood (normal = 850 $\pm$ 150 cells/$mm^3$). The overall information on these individuals is shown in Table 1 below.

Peripheral blood mononuclear cells (PBMCs) were separated from the erythrocytes and the remainder of leukocytes by differential gradient centrifugation. These cells were then suspended in RNAzol™ at 4 x $10^6$ cells/ml and stored frozen in 0.5 ml aliquots. Subsequently, the cells were thawed, and RNA extracted by standard methods. The mRNA transcripts were then used as templates to produce complimentary DNA (cDNA) by reverse transcription using standard methods.

**Example 2: cDNA Analysis**

The CDR3 cDNA length analysis was performed using a two-step PCR. Using the cDNA as template, the TCR BV regions were amplified in a first step by PCR in 24 reactions using primers specific for each of the human BV subfamilies and a BC-specific primer. The sequences of BV and BC primers used were those described in Genevel et al. (Genevel et al., Eur. J. Immunol. 22 : 1261-1269 (1992)), while the sequences of BJ primers were those described in Piusieux et al. (Piusieux et al., J. Immunol. 153 : 2807-2818 (1994)). The BV families are numbered as described by Wei et al., (Wei et al., Immunogenetics 40 : 27-36 (1994)).

**Table 1. HIV+ Study Subject Characteristics**

| Subject | Age | Year of Diagnosis | Antiviral Treatment (duration in months) | CD4+ T Cell | WBC | Lymphs | Monos | PMNs | Eos |
|---|---|---|---|---|---|---|---|---|---|
| | | | | (Cells/mm$^3$) | | | | | |
| 1 | 44 | 1987 | ddC (3) | 280 | 5200 | 1872 | 374 | 2704 | 213 |
| 2 | 28 | 1991 | AZT (12) | 495 | 5600 | 1982 | 672 | 2800 | 84 |
| 3 | 48 | 1987 | AZT (84), ddC (24) | 263 | 3400 | 1020 | 374 | 1938 | 68 |
| 4 | 42 | 1985 | AZT (2) | 270 | 9000 | 2340 | 270 | 6120 | 117 |
| 5 | 48 | 1983 | AZT (24) | 382 | 4800 | 1152 | 288 | 3216 | 77 |
| 6 | 32 | 1989 | AZT (1.5) | 307 | 5600 | 1909 | 560 | 2520 | 56 |
| 7 | 34 | 1987 | AZT (6) | 240 | 5100 | 1377 | 612 | 3009 | 87 |
| 8 | 35 | 1984 | d4T (12) | 476 | 4400 | 1892 | 484 | 1892 | 97 |
| 9 | 33 | 1994 | AZT (12), ddC (2) | 316 | 5300 | 1219 | 636 | 3339 | 58 |
| 10 | 42 | 1986 | AZT (6), d4T (6) | 282 | 5900 | 2313 | 625 | 2773 | 159 |
| 11 | 35 | 1990 | 3TC (8), d4T (3) | 202 | 4700 | 1410 | 470 | 2773 | 71 |
| 12 | 28 | 1993 | ddI (1.5) | 438 | 5700 | 1653 | 399 | 3249 | 342 |
| 13 | 32 | 1989 | d4T (6) | 306 | 6900 | 2346 | 690 | 3381 | 414 |
| 14 | 38 | 1986 | ddI (4), d4T (1) | 473 | 4400 | 1408 | 440 | 2376 | 88 |
| 15 | 37 | 1990 | AZT (50), ddI (50) | 462 | 7200 | 1030 | 828 | 5054 | 209 |
| 16 | 41 | 1989 | d4T (1.5) | 356 | 5600 | 1753 | 515 | 3013 | 230 |
| Mean | 37 | 1988 | | 347 | 5550 | 1667 | 515 | 3131 | 148 |
| SEM | 2 | 1 | | 24 | 326 | 114 | 39 | 268 | 27 |

**Example 3: PCR Reaction**

The PCRs are prepared in a final volume of 50 $\mu$l, with 0.5 $\mu$M of each oligonucleotide primer, 1.5 mM $MgCl_2$, 200 $\mu$M of each dNTP, and 0.125 U of Taq polymerase in the Taq polymerase buffer (Perkin-Elmer, Foster City, CA).

The PCRs are performed in a Perkin-Elmer Gene AMP PCR system 9600, and began with an initial heating at 94°C for 30 sec. followed by 40 cycles of amplification by denaturation at 94°C for 20 sec., annealing of primer at 60°C for 45 sec., and extension of primers at 72°C for 45 sec., with a final extension step at 72°C for 10 min. For each of the 24 BV-BC PCRs, a negative control performed without cDNA was included.

The products amplified in the first step PCR were analyzed in a second step by performing a "run-off" reaction that includes one additional fluorescent primer. Aliquots (2 $\mu$l) of the 24 BV-BC PCRs were subjected to 10 cycles of elongation (run-off) using a nested flurophore-labeled BC oligonucleotide, or in 13 reactions with flurophore-labeled BJ-specific oligonucleotides (0.1 $\mu$M final concentration of the flurophore-labeled primers). The run-off reactions were visualized on 6% acrylamide sequencing gels on a 373 A Applied Systems DNA sequencer with fluorescent size markers (ranging from 80-300 nucleotides in length).

The sizes of the products, which were elongated through the CDR3 regions, giving varying length fluorescent intensity profiles, were analyzed using the Immunoscope software package as described by Pannetier et al. (Pannetier et al., P.N.A.S. (USA) 90 : 4319-4323 (1993); Pannetier et al., Immunol. Today 16 : 176-181 (1993)).

**Example 4: Results from a Normal Individual**

The 24 CDR3 cDNA size patterns, BV1 to BV24, were obtained from T cells from a normal, healthy adult. These patterns exhibited Gaussian distributions and appear to be MHC-independent, as described by Pannetier et al. (Pannetier et al., ImmunologyToday 4: 176-181 (1995) and Pannetier et al., in The human T cell antigen receptor: Selected Protocols and Applications. Ed. Jorge Obsenberg (1996), are depicted in Figure 1. These CDR3 profiles contained fragments 8 to 13 amino acids long, with an average of 10 amino acids. They were stable over time, as well as when tested in separate blood samples taken from the same individual.

**Example 5: Results from HIV+ Patient No. 3**

By contrast to the results described in Example 4 above, the 24 CDR3 profiles from HIV+ Patient No. 3 was distinctly abnormal. The BV4, BV6, BV7, and BV10 patterns appeared as abnormal and putatively oligoclonal, whereas the BV11 and BV24 patterns appeared monoclonal. This can be seen in Figure 2.

**Example 6: Results from HIV+ Patient No. 8**

The 24 CDR3 profiles from Patient No. 8 were found similar to those of Patient No. 3

described in Example 5 above. The BV10, BV22, and BV24 patterns were found to be monoclonal as can be seen in Figure 3.

**Example 7: Results from HIV+Patient No. 9**

The 24 CD3 profiles from Patient No. 9 also appear abnormal. Oligoclonal patterns were found for BV5, BV9, BV13, and BV24, as can be seen in Figure 4.

**Example 8: Results from HIV+Patient No. 15**

The 24 CD3 profiles for Patient No. 15 are shown in Figure 5. Monoclonal patterns were found for BV14, BV22, and BV24, whereas oligoclonal patterns were found for BV6, BV10, BV15, BV16, and BV21.

**Example 9: Results and Comments**

In addition to these oligoclonal and monoclonal patterns of the TCR repertoire, there may also be characteristic lack of expresison of particular BV subtypes. For example, there is lack of expression of BV12 in 4 of the 4 patient samples tested, and in 3 of 4 patient samples tested. This may be seen in Table 2 below.

When a particular clonal pattern is identified as indicative of a disease or condition, e.g., infection with a particular microbe, such as the monoclonal pattern for BV24 in HIV infection, the unique nucleotide sequence from the monoclonal CDR3 region that is formed by the recombination of nucleotides contributed by the V gene region, the D gene region, and the J gene region is thus, used to create a disease-specific probe or primer that is used in diagnosis, prognosis and/or therapy. An example of this is in Table 3 below.

**Example 10: Effect of IL-2 Therapy on HIV+ Subject No. 8**

There also may occur changes in the TCR repertoire expression patterns as a consequence of therapeutic intervention. For example, Patient No. 8 underwent immunotherapy with ultralow dose IL-2 for 6 months, and the BV12 pattern changed from lack of expression before therapy to expressing of this BV subtype after therapy. (see Table 2 below). This shift in the profile shows a normalization of the patient's TCR repertoire.

**Table 2: BV Expression of Selected Subjects**

| | | BV REGION NUMBER | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient No. | Time (mths) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 |
| 3 | 0 | X | X | X | X | X | X | X | X | X | X | | | X | X | X | X | X | X | X | | X | X | X | X |
| 8 | 0 | X | X | X | X | X | X | X | X | X | X | | | X | X | X | X | X | X | X | | X | X | X | X |
| 9 | 0 | | X | X | | X | X | X | X | X | X | X | | X | X | X | X | X | X | X | | X | X | X | X |
| 15 | 0 | X | X | X | X | X | X | X | X | X | X | X | | X | X | X | X | X | X | X | X | X | X | X | ? |
| 8 | 6 | X | X | X | X | | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |

**Example 11: cDNA Sequences of BV Monoclonal cDNA Pattern**

A particular clonal pattern was identified as indicative of desease, e.g., with a particular microbe, the monoclonal pattern for BV24 in HIV infection, which was exhibited by all HIV+ subjects tested.

The cDNA fragment was sequenced, and its sequence is provided in Table 3 below.

**Table 3: cDNA Sequence for BV24 Monoclonal Pattern Peak**

| | | | | | |
|---|---|---|---|---|---|
| GATGCCATGG | TCATCCAGAA | CCCAAGATAC | CAGGTTACCC | CATTTGGAAA | 50 |
| GCCAGTGACC | CTGAGTTGTT | CTCAGACTTT | GAACCATAAC | GTCATGTACT | 100 |
| GGTACCAGCA | GAATGTCAAGT | CAGGCCCCAA | AGCTGCTGTT | CCACTACTAT | 150 |
| GACAAAGATT | TTAACAATGA | AGCAGACACC | CCTGATAACT | TCCAATCCAG | 200 |
| GAGGCCGAAC | ACTTCTTTCT | GCTTTCTTGA | CATCCGCTCA | CCAGGCCTGG | 250 |
| GGGACGCAGC | CATGTACCTG | TGTGCCACCA | GCAGAGTAGA | GGGAGATGAG | 300 |
| CAGTTCTTCG | GGCCAGGGAC | ACGGCTCACC | GTGCTAGAGG | ACCTGAAAAA | 350 |
| CGTGTTCCCA | CCC . . . . . | | | 363 | |

| | |
|---|---|
| 722 | Total Length |
| 1- 181 | V-beta 24 region. |
| 282-300 | D-beta/CDR3 region. |
| 296-336 | J-beta 2.1 region. |
| 337-onward | Constant Beta region. |

The unique nucleotide sequence from the monoclonal CDR3 region that is formed by the recombination of nucleotides contributed by the V gene region, the D gene region, and the J gene region can be used to create disease-specific probes and/or primers for use in diagnosis, prognosis and/or therapy. For example, the sequence of the monoclonal BV24 pattern expressed by Patient No. 3 is shown in Table 3 above. The nucleotides contributed by the V gene region are believed to include positions 1 to 181, the the D gene region 182 to 300, and by the J gene region 196 to 331.

Having now generally described the invention, as well as by reference to the examples, an artisan will understand that many variations may be applied that are within the confines of the invention.

## CLAIMS

1. A method of diagnosing a condition or disease associated with microbial infections, congenital or acquired immunodeficiencies, inflammatory, auto-immune, allergic, or dermatologic diseases, sarcoidosis, immunoscenesence, sepsis, tissue necrosis, malignancies, or vaccine administration, comprising

(a) obtaining a sample comprising T or B cells from a subject suspected of being afflicted with a condition selected from the group consisting of conditions associated with microbial infections by congenital or acquired immunodeficiencies, inflammatory, auto-immune, allergic, or dermatologic diseases, sarcoidosis, immunoscenesence, tissue sepsis, necrosis, malignancies, or vaccine administration;

(b) reverse transcribing the sample's T or B cell receptor (TCR and BCR) mRNA fragments into cell receptor (CR) cDNA fragments prior to the determining step;

(c) determining the subject's most abundant length CR cDNA fragment, at least one of the CR cDNA fragments sequences, their length distribution or curve, and/or the area thereunder;

(d) comparing either the most abundant length CR cDNA fragment, at least one of the CR cDNA fragments sequences, their length distribution or curve, and/or area thereunder, to a library comprising either the most abundant length CR cDNAs, at least one of the CR cDNA fragment sequences, their length distributions or curves, areas thereunder, prognosis thereof, and/or diagnosis thereof, for all normal and diseased subject types of the same species, to determine any similarities; and

(e) diagnosing a disease or condition when either the patient's most abundant length CR cDNA fragment, at least one of the CR cDNA fragments sequences, their length distribution or curve, and/or area thereunder, is similar to either one of the most abundant length CR cDNA fragments, at least one of the CR cDNA fragments sequences, their length distribution or curve, and/or area thereunder, corresponding to a specific condition or disease comprised by the library.

2. The method of claim 1, wherein the library comprises a plurality of disease libraries, each library comprising either the most abundant length CR cDNA fragments, at least one of the CR cDNA fragments sequences, their length distribution or curve, and/or area thereunder, for all normal and specific diseased subject types.

3. The method of claim 1, wherein the library further comprises a library of either the most abundant length CR cDNA fragment, at least one of the cDNA fragments sequences, their length distributions or curves, and/or area thereunder, for all normal subject types, each of which is afflicted by a plurality of diseases.

4. The method of claim 1, wherein the library comprises a prognosis library

comprising either the most abundant length CR cDNAs, at least one of the CR cDNA fragments sequences, their length distributions or curves, or areas thereunder, and/or prognosis thereof, for all normal and diseased subject types from the same species.

5. The method of claim 1, wherein the mRNA comprises TCR BV mRNA, and the library comprises TCR BV cDNA.

6. The method of claim 1, wherein the mRNA is selected from the group consisting of TCR AV, CV, and DV mRNA, and the library comprises cDNA selected from the group consisting of TCR AV, CV, and DV cDNA.

7. The method of claim 1, wherein the mRNA comprises BCR VH mRNA, and the library comprises BCR VH cDNA.

8. The method of claim 1, wherein the mRNA comprises BCR VL mRNA, and the library comprises BCR VL cDNA.

9. The method of claim 1, wherein the sample comprises serum or lymphatic fluid.

10. The method of claim 1, wherein the species comprises an animal selected from the group consisting of domestic and wild animals.

11. The method of claim 10, wherein the animal is a mammal.

12. The method of claim 11, wherein the animal comprises a human.

13. The method of claim 1, wherein the microorganism is selected from the group consisting of viruses, bacteria, fungi, mycoplasm, and parasites.

14. The method of claim 13, wherein the subject is HIV seropositive.

15. The method of claim 1, wherein the most abundant length CR cDNA, its length distribution, or curve and/or area thereunder is obtained by

separately amplifying the transcribed CR cDNA by the polymerase chain reaction (PCR) with unlabeled primers complementary to regions vicinal to the complementarity determining regions of the desired T cell receptor (TCR) chain up- and down-stream thereto, under conditions effective to attain full saturation;

amplifying the thus obtained unlabeled cDNA by PCR with one additional primer comprising a first label, under conditions effective to attain a run-off reaction elongating the amplified cDNA over the vicinal region; and

separating the labeled cDNA and analyzing the most abundant length CR cDNA, its length distribution or curve, and/or the area thereunder.

16. The method of claim 15, wherein the CR comprises the TCR, the desired region comprises the BV region, and the primers are selected from the group consisting of the BV and BC regions, and the BV and BJ regions.

17. The method of claim 16, wherein the desired BV region comprises a CDR3, and the BV, BC, or BJ primers comprise sequences vicinal to the CDR3 region.

18. The method of claim 15, wherein the CR comprises the BCR, the desired region comprises the VH region, and the primers are selected from the group consisting of the VH and CH regions, and the VH and JH regions.

19. The method of claim 18, wherein the desired VH region comprises a CDR3, and the VH, CH, or JH primers comprise sequences vicinal to the CDR3 region.

20. The method of claim 15, wherein the amplification step is separately repeated with different primers and labels, and the differently labeled DNAs are separated on a gel, and analyzed as described.

21. The method of claim 20, wherein the separation of the cDNA is attained by gel electrophoresis, and the most abundant length cDNA, its length distribution or curve, and/or areas thereunder are analyzed by a computer means.

22. The method of claim 21, wherein the comparison of the subject's CR cDNA information to the library standards is computerized.

23. The method of claim 1, wherein steps (b) to (e) are automated.

24. A method of assessing the prognosis of a subject afflicted with a condition associated with microbial infections, congenital or acquired immunodeficiencies, inflammatory, auto-immune, allergic, or dermatologic diseases, sarcoidosis, immunoscenesence, sepsis, tissue necrosis, malignancies, and vaccine administration, comprising

(a) obtaining a sample comprising T or B cells from a subject afflicted with a condition selected from the group consisting of conditions associated with microbial infections, congenital or acquired immunodeficiencies, inflammatory, auto-immune, allergic, or dermatologic diseases, sepsis, tissue necrosis, malignancies, immunoscenesence, sarcoidosis, and vaccine administration;

(b) reverse transcribing the sample's T (TCR) or B (VCR) cell receptor (CR) mRNA into CR cDNA;

(c) determining the subject's most abundant length CR cDNA fragment, at least one of the CR cDNA fragments sequences, their fragment length distribution or curve, and/or area thereunder;

(d) comparing either the most abundant length CR cDNA fragment, at least one of the CR cDNA fragments sequences, their length distribution or curve, and/or area thereunder, to a library comprising either the most abundant length CR cDNA fragments, at least one of the CR cDNA fragments sequences, their length distributions or curves, area thereunder, diagnosis thereof, and/or prognosis thereof, obtained from all normal and diseased subject types from the same species,

to determine any similarities;

(e) diagnosing a disease or condition when either the patient's most abundant length CR cDNA fragment, at least one of the CR cDNA fragments sequences, their length distribution or curve, and/or the area thereunder, is similar to either the most abundant length CR cDNA fragment, at least one of the the CR cDNA fragments sequences, their length distribution or curve, and/or area thereunder, corresponding to a specific disease or condition, comprised by the library; and

(f) assessing a specific prognosis for the subject when, either the patient's most abundant length CR cDNA fragment, at least one of the CR cDNA fragments sequences, their distribution or curve, and/or the area thereunder, upon a comparison with the CR library, is found to be similar to either one of the most abundant length CR cDNA fragments, at least one of the CR cDNA fragments sequences, their length distribution or curve, and/or area thereunder, for a specific disease or condition CR cDNA subject type with a specific prognosis.

25. A method of assessing the progress of treating for a subject afflicted with a condition associated with microbial infections, congenital or acquired immunodeficiencies, inflammatory, auto-immune, allergic or dermatologic diseases, sarcoidosis, immunoscenesence, sepsis, tissue necrosis, malignancies, and vaccine administration, comprising

(a) obtaining samples comprising T or B cells from a subject afflicted with a condition selected from the group consisting of conditions associated with infection by a microorganism, congenital or acquired immunodeficiencies, inflammatory, auto-immune, allergic, or dermatologic diseases, sepsis, tissue necrosis, sarcoidosis, immunoscenesence, malignancies, and vaccine administration, prior to initiating treatment, and at specific time intervals thereafter;

(b) reverse transcribing the samples' T (TCR) or B (BCR) cell receptor (CR) mRNA into CR cDNA;

(c) determining the subject's most abundant length CR cDNA fragment, at least one of the CR cDNA fragments sequences, thier length distribution or curve, and/or area thereunder, prior to starting therapy and at the time intervals thereafter;

(d) comparing either the most abundant length CR cDNA fragment, at least one of the CR cDNA fragments sequences, their length distribution or curve, and/or area thereunder, obtained prior to initiation of the treatment with that (those) obtained at a later time interval (s), and to a CR cDNA library comprising either the most abundant length CR cDNA fragments, at least one of the CR cDNA fragments sequences, their length distributions or curves, and/or area thereunder, for all normal and diseased subject types from the same species, to determine any variations; and

(e) assessing an effective treatment when, as time progresses, either the patient's most abundant length CR cDNA fragment, at least one of the cDNA fragments sequences,

their distribution or curve, and/or area thereunder, becomes more similar to either the most abundant length CR cDNA, at least one of the cDNA fragments sequences, their length distribution or curve, and/or area thereunder, for the corresponding normal CR cDNA subject type or less similar to that obtained prior to initiation of treatment or to the CR cDNA for the specific condition or disease.

26. A diagnostic kit, comprising

a library of all normal subject types T (TCR) or B (BCR) cell (CR) cDNAs for normal and diseased standards; and

instructions for its use.

27. The kit of claim 26, further comprising

reverse transcriptase; and

PCR reagents.

28. The kit of claim 26, wherein

the library is in computerized form.

29. The kit of claim 26, wherein

the standards are selected from the group consisting of the most abundant length T (TCR) or B (BCR) cell receptor (CR) cDNA fragments, at least one of the CR cDNA fragments sequences, their length distributions or curves, areas thereunder, and prognosis thereof, for all normal and diseased subject types.

30. The kit of claim 26, wherein the library comprises TCR cDNA sequences.

31. The kit of claim 30, wherein the TCR cDNA library comprises TCR BV cDNA sequences.

32. The kit of claim 30, wherein the library comprises the most abundant length BCR cDNA fragments sequences.

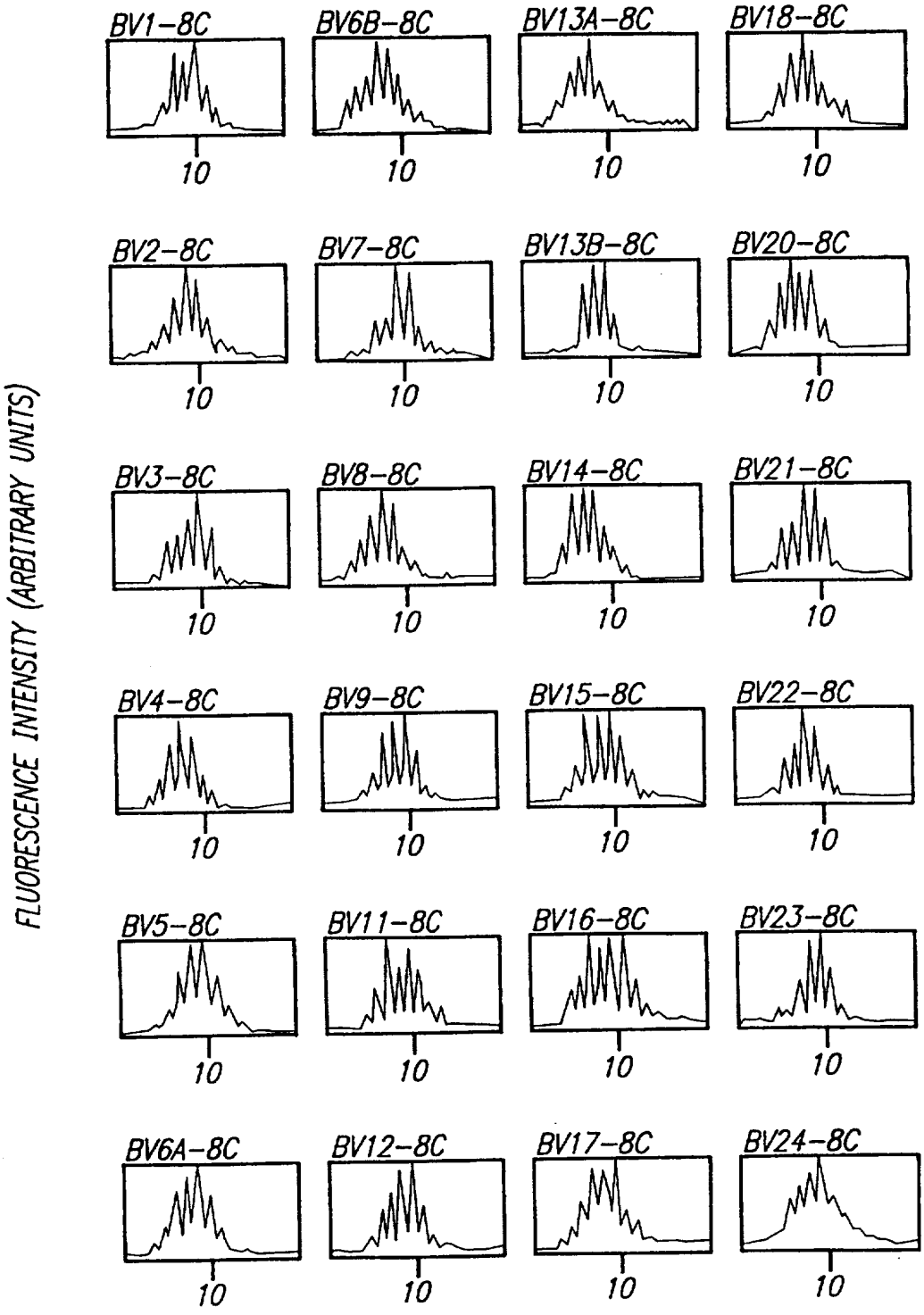
33. The kit of claim 32, wherein the library comprises the most abundant length BCR BV cDNA fragments sequences.

34. The kit of claim 26 wherein the library comprises standards for a plurality of subject's species.

35. The kit of claim 26, wherein the library comprises standards for a plurality of diseases or conditions.

36. The kit of claim 26, further comprising PCR reagents, and primers for regions vicinal to the CDRs.

37. The kit of claim 26, further comprising one or more DNA labels selected from the group consisting of phosphorescent, radioactive, and fluorescent labels.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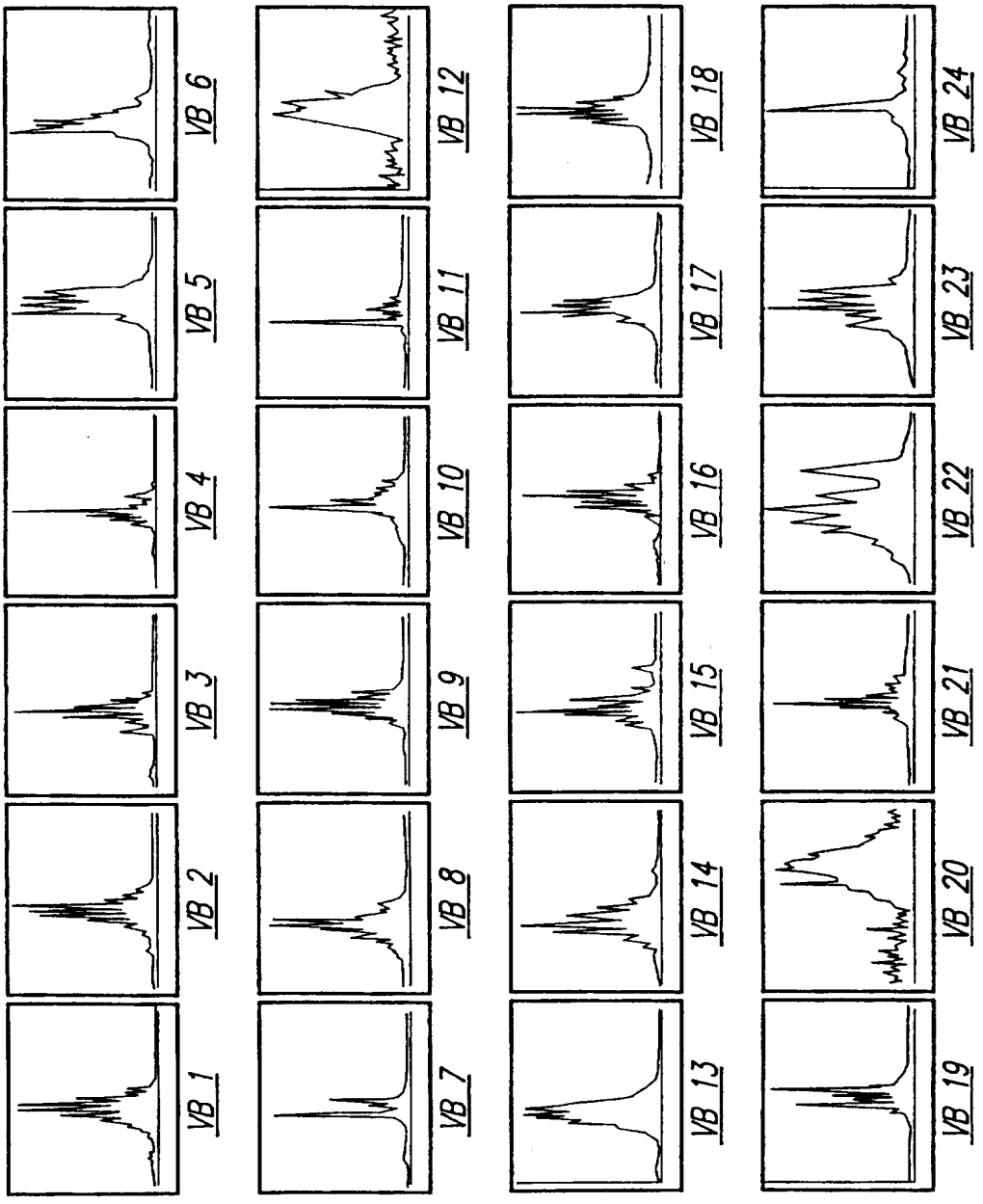
TCRB CDR3 LENGTH (aa)

FIG. 1

FIG. 2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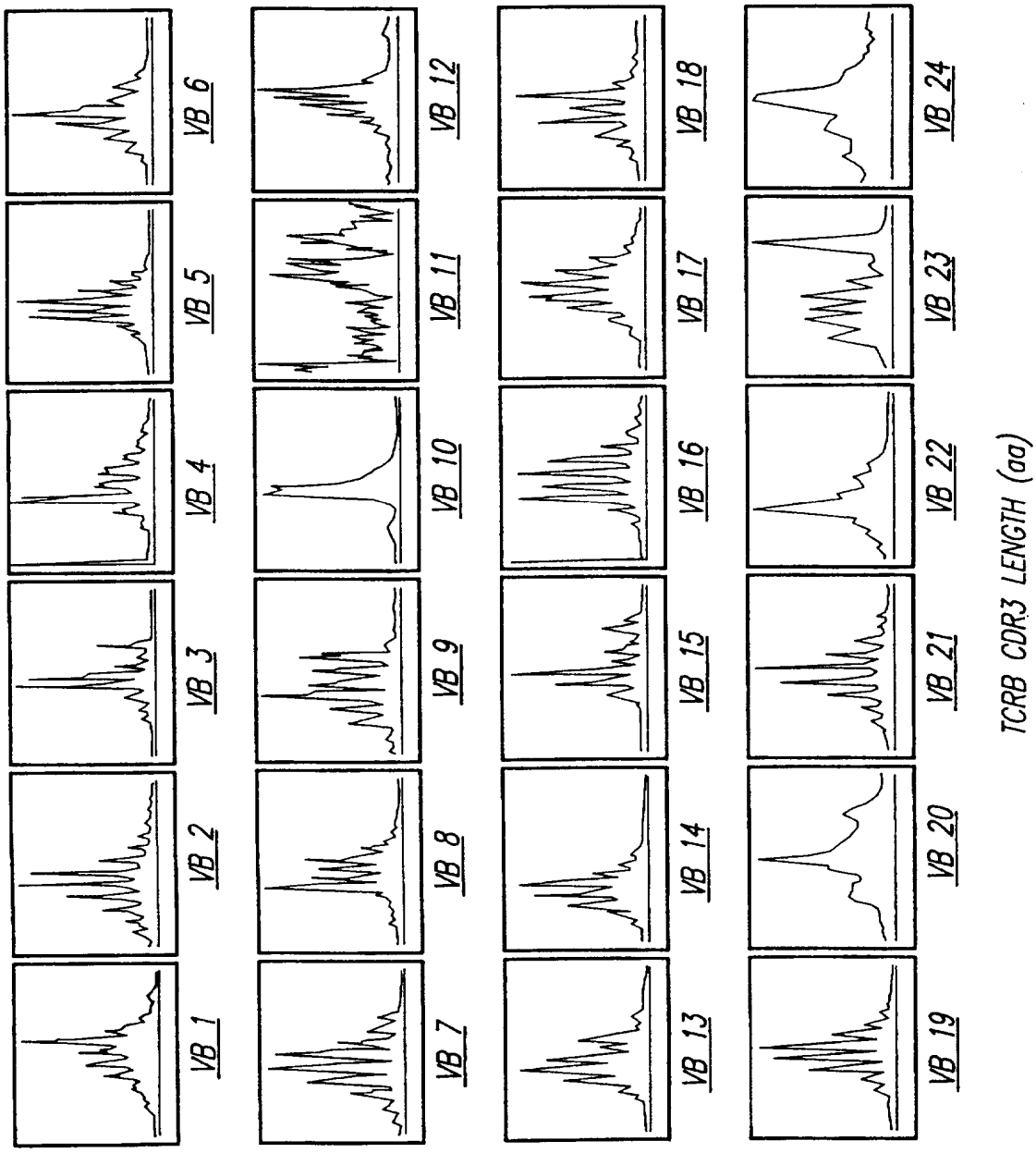
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FIG. 3

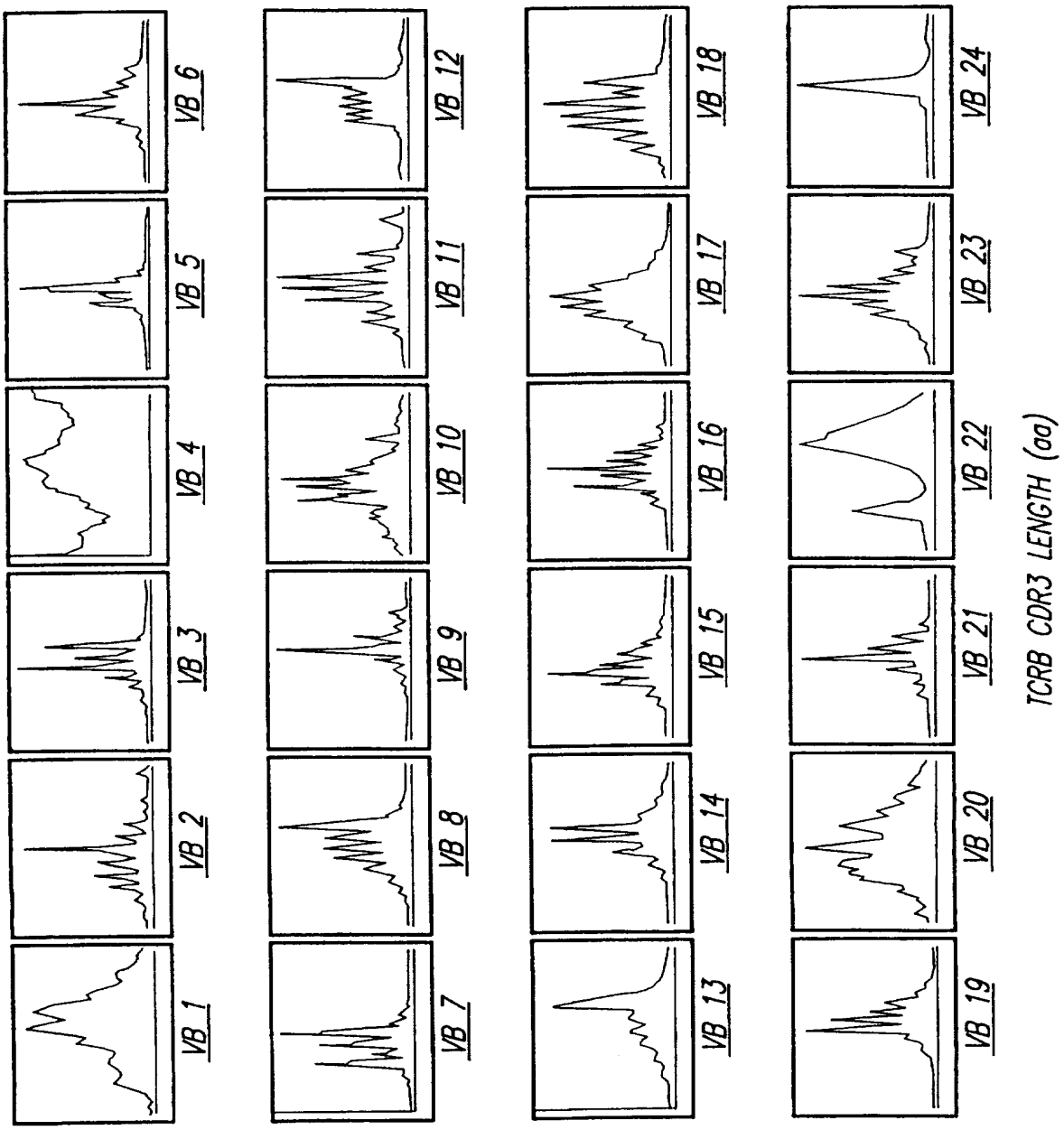
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FIG. 4

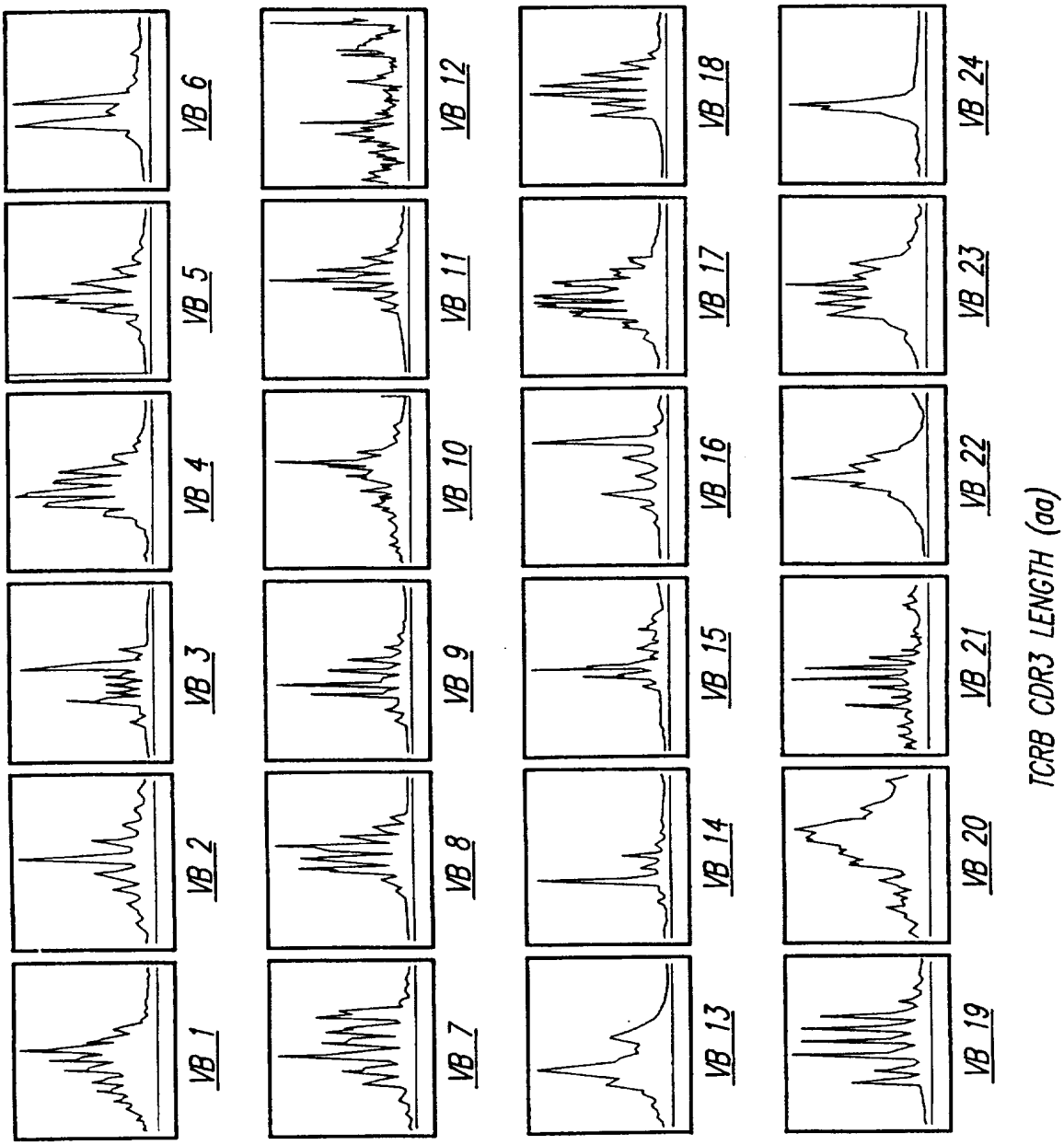
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FIG. 5

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**(71) Applicant** *(for all designated States except US)*: CORNELL RESEARCH FOUNDATION, INC. [US/US]; Suite 105, 20 Thornwood Drive, Ithaca, NY 14550 (US).

**(72) Inventor; and**
**(75) Inventor/Applicant** *(for US only)*: SMITH, Kendall, A. [US/US]; Apartment 3A, 1075 Park Avenue, New York, NY 10128 (US).

**(74) Agents:** AMZEL, Viviana et al.; Pretty, Schroeder & Poplawski, Suite 2000, 444 South Flower Street, Los Angeles, CA 90071 (US).

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**(54) Title:** IDENTIFICATION OF ABNORMALITIES IN THE EXPRESSION OF T AND B CELL ANTIGEN RECEPTORS AS DISEASE INDICATORS

**(57) Abstract**

A method for diagnosing a disease, assessing the prognosing and assessing the progress of a treatment for a disease are based upon a comparison between the T Cell receptor or B cell receptor mRNA expression in a patient suffering from a disease ant the T cell receptor or B cell receptor mRNA expression in a cDNA library for all normal and diseased subject types of the same species in order to determine any similarities. The mRNAs are reverse transcribed to cDNA fragments, and diagnosis, prognosis and treatment assessment occurs when a cDNA from the patient is similar a cDNA fragment known to correspond to a particular disease.

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# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/10557

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :C12Q 1/68; C12P 19/34
US CL :435/6, 91.2
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 91.2; 935/77, 78

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS, MEDLINE, BIOSIS
search terms: T cell receptors, B cell receptors, mRNA, disease, diagnose, expression

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | AUWAERTER, P.G. et al., "Changes within T cell Receptor VB Subsets in Infants Following Measles Vaccination", Clinical Immunology and Immunopathology. May 1996, Vol 79. No. 2. pages 163-170, especially 165,167-169. | 1-37 |
| Y,P | AHANGARI et al. RT-PCR Topology of Chronic Psoriasis Skin Based on Analysis of T-Cell Receptor B VArilable Region Gene Usage. Scandavanian J. Immunology. January 1997, Vol. 45, pages 534-540, especiallt pages 534-535. | 1-37 |

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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Date of the actual completion of the international search
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/10557

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
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| Y | GULWANI-AKOLKAR et al. VB-Specific Changes in the T-Cell Receptor Repertoires of Lamina Propia Lymphocytes in Crohn's Disease. Annals of the New York Academy of Sciences. 1995, Vol. 756, pages 403-405, see entire document. | 1-37 |
| Y | TSAI et al. Analysis of rearranged T Cell Receptor (TCR) VB Transcripts in Livers of Primary Biliary Cirrhosis: Preferential VB Usage Suggests Antigen-Driven Selection. Clinical and Experimental Immunology. January 1996, Vol. 103, No. 1, pages 99-104, especially pages 100-102. | 1-37 |
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